Conversion of hepatitis B virus relaxed circular to covalently closed circular DNA is supported in murine cells

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Materials and Methods

Cell lines and animals. The murine Hepa1.6, AML12, and human NTCP expressing HepG2 cell lines were maintained in Dulbecco's modified Eagle medium (DMEM; Thermo Fisher Scientific) supplemented with 10% (vol/vol) fetal bovine serum (FBS, Hyclone), 100 units/ml of penicillin, and 100 µg/ml streptomycin (Thermo Fisher).

NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}/SzJ (NRG) mice were obtained from The Jackson Laboratory (catalog number 007799).

Generation and purification of recombinant cccDNA. Recombinant HBV cccDNA (Rccc) contains a 39 bp insertion (CCCCAACTGGGGTAACCTTTGGGCTCCCCGGGCGCGACC) in the polymerase domain, which does not lead to a frame shift. RcccDNA was generated as previously described[38,46]. Briefly, the parental Rccc production plasmid containing the HBV genotype D genome (GenBank accession number: U95551.1) was amplified in the DNA methylation deficient *E.coli* strain ZYCY10P3S2T *dam-*/*dcm-*, which was subsequently treated with L-arabinose (Sigma Aldrich) at a final concentration of 0.01% (w/vol) to induce generation of HBV RcccDNA. RcccDNA was further purified by NucleoBond Maxi prep kit (Macherey Nagel).

Generation of recombinant rcDNA and NeutrAvidin-RrcDNA complex.
The method to generate recombinant rcDNA (RrcDNA) and NeutrAvidin-RrcDNA complex has been previously described in detail[38]. Briefly, the minus-strand ssDNA and plus-strand ssDNA were mixed at a 1:1 molar ratio in buffer containing 500 mM NaCl and subsequently annealed by incubating at 95˚C for 3 min and decreasing incubation temperature to 25˚C at a rate of 1˚C/min using a thermocycler. The annealing product was subsequently purified by agarose gel electrophoresis. To generate RrcDNA, the precursor was incubated and ligated with oligo PU-O-5573 (5' Biotin-GAAAAAGTTGCATGGTGCTGGTG, Integrated DNA Technologies) and oligo PU-O-5670 (5' rGrCrArArCrUrUrUrUrUrCrArCrCrUrCrUrGrCACGTCGCATGGAGACCACCGT, Integrated DNA Technologies) to restore the flap structure and RNA primer on the minus and plus-strands, respectively. To generate the NeutrAvidin RrcDNA complex, RrcDNA was incubated with NeutrAvidin (Thermo Fisher) at a final concentration of 4 mg/ml in buffer containing 20 mM HEPES-KOH (pH 8.0) and 50 mM KCl at 37˚C for 30 min.

Transfection of rcDNA DNA and GFP encoding plasmid into murine cell lines.
Murine and human hepatoma cells were seeded in collagen-coated 96 well plates at a density of 2.5 x 10^4 cells/well. Cells were transfected 24 hours after plating, using 50 ng/well of RrcDNA DNA or GFP encoding plasmid.

Hydrodynamic delivery of recombinant cccDNA and rcDNA DNA into mice. Hydrodynamic delivery of recombinant cccDNA and rcDNA was performed as previously described [29]. Briefly, a total of 10 µg of recombinant of cccDNA or rcDNA was injected into the tail vein of NRG male or female mice in a volume of saline equivalent to 8% of the body mass of the mouse (e.g., 2 ml for a mouse of 25 g). The total volume was delivered within 5–8 seconds. All protocols and procedures have been reviewed and approved by the Princeton University Institutional Care and Use Committee (registration #1930).

Histology processing and multiplex fluorescent immunohistochemistry Tissue samples were fixed for a minimum of 72 hours in 4% paraformaldehyde before being processed in a Tissue-Tek VIP-5 automated vacuum infiltration processor (Sakura Finetek USA) and embedded in paraffin using a HistoCore Arcadia paraffin embedding machine (Leica). 5 µm tissue sections were generated using a RM2255 rotary microtome (Leica) and transferred to positively charged slides, deparaffinized in xylene, and dehydrated in graded ethanol. A Ventana Discovery Ultra (Roche) tissue autostainer was used for multiplex fluorescent immunohistochemistry (fmIHC). In brief, tyramide signaling amplification (TSA) was used in an iterative approach to covalently bind Opal fluorophores (Akoya Bioscience) to tyrosine residues in tissue, with subsequent heat stripping of primary-secondary antibody complexes until all three antibodies were developed. Livers from wild-type NRG mice were used as a negative control for HBcAg. CD68 and CK17/19 specificity was confirmed via specificity to sinusoidal macrophages and biliary epithelium respectively. Specific details for the three plex fmIHC are outlined in **Table. S2**. All primary antibodies were of rabbit origin, and thus developed with a secondary goat anti-rabbit HRP-polymer antibody (Vector Laboratories) for 20 min at 37˚C. All Opal TSA-conjugated fluorophore reactions took place for 20 min. Slides were counterstained with spectral DAPI (Akoya Biosciences) for 16 min before being mounted with ProLong gold antifade (ThermoFisher).

Multispectral microscopy. Fluorescently labeled slides were imaged using a Vectra Polaris TM Quantitative Pathology Imaging System (Akoya Biosciences). Exposures for all Opal dyes on the Vectra were set based upon regions of interest with strong signal intensities to minimize exposure times and maximize the specificity of signal detected. Whole slide images were imported directly into HALO (Indica Labs) for image analysis.

Quantitative analysis of multiplex fluorescent immunohistochemistry. View settings were adjusted to allow for optimal visibility of immunomarkers and to reduce background signal by setting threshold gates on minimum signal intensities. Next, slides were manually annotated so analysis was conducted exclusively on liver by excluding the background. For quantifying the absolute number and overall percentage of hepatocytes with HBcAg, we utilized the Halo (Indica Labs) HighPlex phenotyping modules (v4.0.4). In brief, this algorithm was used to first segment all cells within the annotated liver sections using DAPI counterstain. Thresholds for nucleus size were set at 15 micrometers squared, as to exclude most smaller nuclei, such as those of sinusoidal macrophages, biliary epithelium, and endothelium. Next, minimum cytoplasm and membrane thresholds were set for each fluorophore to detect positive staining within each of the segmented cells. Parameters were set using the real-time tuning mechanism that was tailored for each individual sample based on signal intensity. Phenotypes of either HBcAg+ or HBcAg- hepatocytes were determined by selecting inclusion and exclusion parameters as follows respectively: CK17/19-CD68-HBcAg+ or CK17/19-CD68-HBcAg-. The algorithm yielded outputs of both total cell counts and percentage of hepatocytes with HBcAg. The quantitative output for the HighPlex was exported as a .CSV file and uploaded into GraphPad Prism (v9.2.0) for statistical analysis with a One-Way ANOVA followed by a Tukey post hoc.

Purification of HBV DNA and RNA from mouse serum. HBV DNA and RNA from serum were purified by QIAamp MinElute Virus spin kit (Qiagen) per the manufacturer's instructions. Briefly, 10 µl serum was diluted to 50 µl with PBS and digested with 25 µl of proteinase K in AL lysis buffer (Qiagen) at 56 °C for 30 min. 250 µl of 100% (vol/vol) ethanol was subsequently added and the suspension was applied to a QIAamp DNA mini kit column and centrifuged for 1 min at 6,000 x g. After washes with buffers AW1 and AW2,

HBV DNA and RNA was eluted in 20 µl of nuclease free H_2O. 10 µl of each sample was treated with RNAse free DNase I (Qiagen) to obtain RNA samples.

Purification of HBV DNA and RNA from mouse liver tissues. Purification of HBV DNA and RNA from mouse liver tissues was performed using Quick-DNA/RNA Microprep Plus Kit (Zymo Research) per manufacturer's instructions. Briefly, stainless steel beads (5 mm, Qiagen) were added to 20-25 mg section of liver tissue mixed with DNA/RNA shield buffer (provided by the kit). Liver tissues were subsequently homogenized via TissueLyzerII (Qiagen) for 3 cycles at a setting of 50 oscillation/s, 2 min/cycle. The samples were then centrifuged to remove cell debris and treated with protease K at 55˚C for 30 min. Tissue DNA was subsequently purified by Zymo-Spin IC-XM column provided by the kit, while the flowthrough RNA was purified by Zymo-Spin IC column provided by the kit. On column DNase digestion was performed to remove residual DNA contaminations from the RNA samples. Both HBV DNA and RNA were eluted in 30 µl of RNase-free water and stored at -80˚C.

Determination of HBV DNA by quantitative PCR. To amplify HBV DNA, the following primers were used: GGAGGCTGTAGGCATAAATTGG (forward primer), and CACAGCTTGGAGGCTTGAAC (reverse primer). Primers were used at a concentration of 500 nM in a 20 µl reaction system containing 2 µl of HBV DNA or standard and 1x SYBR Green PCR master mix (Thermo Fisher). The following PCR program was run on a Step One Plus qPCR machine (Life Technologies): 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

Determination of pgRNA by reverse transcription (RT)-quantitative PCR. HBV pgRNA purification from mouse serum and liver tissue was performed by two-step RT-quantitative PCR. First, 8 µl RNA samples (from serum) or 2 µg of RNA (from liver tissue) were used for reverse transcription using MultiScribe reverse transcriptase (Thermo Fisher). The reverse transcription primer (TCTCACACCGTAACACACGACACAGGCGAGGGAGTTCTTCTTCTA) contains a non-viral tag sequence attached at the 5' end of the strand-specific HBV sequence that was used to prime the reverse transcription reaction. The reaction was assembled with 1X first-strand buffer, 10 mM dithiothreitol (DTT), 0.5 mM deoxynucleoside triphosphates (dNTPs), 250 nM RT primer, 0.4 U/µl recombinant RNase inhibitor (Takara), and 1.25 U/µl of MultiScribe reverse transcriptase (Thermo Fisher) in a volume of 20 µl. The mixture was incubated at 42°C for 50 min and then 70°C for 15 min. The mixture was subsequently diluted to a final volume of 40 µl with nuclease-free water for real-time qPCR. Real-time qPCR was prepared using TaqMan universal PCR mix (Applied Biosystems) and a FAM-BHQ probe. Briefly, 2 µl of diluted cDNA was mixed with primers (forward primer AGACCACCAAATGCCCCT and reverse primer TCACACCGTAACACACGACAC) at a final concentration of 300 nM and detection probe (FAM 5' CAACACTTCCGGAGACTACTGTTGTTAGACG 3' BHQ1) at a final concentration of 200 nM. The reaction mixtures were then subjected to 40 cycles of PCR, with each cycle consisting of 15 s at 95°C and 1 min at 60°C.

Determination of HBV cccDNA by quantitative PCR. HBV cccDNA was purified from cells using the Hirt extraction method, as previously described (38). Cells were washed twice with 1x PBS and subsequently lysed in buffer containing 10 mM Tris-HCl, pH 8.0, 0.625% SDS, and 10 mM EDTA. The cell lysate was incubated for 10 min at 20 °C and transferred to a clean Eppendorf tube. NaCl was added to the lysate to a final concentration of 1 M and incubated overnight at 4 °C. The lysate was then clarified by

centrifugation at 20,000g at 4 °C, the supernatant was transferred to a clean Eppendorf tube and extracted twice with phenol and once with phenol–chloroform (1:1, vol/vol). The aqueous phase was collected, and the DNA was precipitated and washed with ethanol. The DNA was then dissolved in 20 µl nuclease free water. 1000 ng of DNA eluent were treated with 5 U of T5 exonuclease (NEB) for 1 hr at 37˚C in a final volume of 10 µl. T5 exonuclease was inactivated at 75˚C for 15 min. To amplify HBV DNA, T5 treated DNA samples were diluted 4x in H2O, and 2 µl DNA was used in each qPCR reaction. The following primers were used: GGAGGCTGTAGGCATAAATTGG (forward primer), and CACAGCTTGGAGGCTTGAAC (reverse primer). Primers were used at a concentration of 500 nM in a 12 µl reaction system containing 2 µl of T5 treated DNA or standard and 1x SYBR Green PCR master mix (Thermo Fisher). The following PCR program was run on a Step One Plus qPCR machine (Life Technologies): 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

HBV cccDNA purification from mice liver tissues and detection by Southern blotting. Snap frozen mice liver tissues was thawed and cut into small pieces. Solution containing 10 mM Tris–HCl, pH 7.5 and 10 mM EDTA was added to the liver tissue, which was then homogenized by a Dounce homogenizer. The lysate was then centrifuged at 2,500 rpm for 15 min and the pellet was collected and resuspended in 1 ml lysis buffer (10 mM Tris-HCl, pH 8.0, 0.625% SDS, and 10 mM EDTA). The lysate was kept at room temperature for 30 min, and 5 M NaCl was added to the lysate to reach a final concentration of 1 M. The lysate was incubated at 4℃ overnight and subsequently centrifuged at 12000 × g for 30 min at 4℃. The supernatant was collected and extracted by phenol (pH 8.0) twice and phenol-chloroform (pH 8.0, 1:1) once. The supernatant was then supplemented with 0.8 volume of isopropanol and kept at -20℃ overnight. The solution was then spun at 12,000 × g at 4˚C for 30 min, the pellet was washed by 70% ethanol, dried for 5 min, and dissolved in nuclease-free water. For qPCR reaction, 4000 ng of purified HBV cccDNA was treated with 10 U of T5 exonuclease at 37˚C for 1 hr, and T5 exonuclease was inactivated by incubation at 75˚C for 15 min. Detection of cccDNA by PCR was performed as described in the 'Determination of HBV cccDNA by quantitative PCR' section. HBV cccDNA detection by Southern blotting was carried out as previously described[38].

Preparation of murine (Hepa1.6 and AML12) and human (hNTCP-HepG2) cell nuclear extracts. Murine and human cell nuclear extracts were prepared as previously described[38]. Briefly, cells were harvested, resuspended in hypotonic buffer (10 mM HEPES-KOH (pH 8.0), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM DTT, 0.5 mM PMSF and 1x Protease Inhibitor Cocktail (Sigma Aldrich) and lysed using a Dounce homogenizer (DWK). The lysed cells were then spun at 1,500 x g for 5 min. The pellet (the nuclear fraction) was resuspended in high salt solution containing 20 mM HEPES (pH 8.0), 1.5 mM $MgCl_2$, 700 mM KCl, 5% (vol/vol) glycerol, 0.5 mM PMSF, and 1x Protease Inhibitor Cocktail (Sigma Aldrich). The mixture was incubated at 4˚C for 30 min before being subjected to centrifugation at 20,000 x g for 30 min. The supernatant was recovered, concentrated, and dialyzed against buffer containing 20 mM HEPES-KOH (pH 8.0), 50 mM KCl, 5% glycerol, 1 mM DTT, and 0.5 mM PMSF. The final protein concentrations of nuclear extracts were between 15-30 mg/ml.

Purification of recombinant proteins. Purification of recombinant murine and human PCNA, RFC complex, POLδ complex, LIG1, and FEN-1 have been previously described in detail with minor modifications[38]. RFC and POLδ complex were both purified from Sf9

insect cells. 6xHis and Flag tags were included to facilitate purification and increase purity by Ni and anti-Flag antibody conjugated resins (Sigma Aldrich).

cccDNA formation assay in murine and human nuclear extracts. The reaction has been previously described[38]. Briefly, RrcDNA or NA-RrcDNA substrates (29 fmol) were incubated with murine or human cell nuclear extracts supplemented with 20 mM Tris-HCl (pH 7.6), 50 mM KCl, 0.1 mM dNTP, 5 mM $MgCl_2$, 1 mM reduced glutathione, 2.6 mM ATP, 26 mM phosphocreatine disodium (Sigma Aldrich), and 6 μg/ml creatine phosphokinase (Sigma Aldrich). The mixture was incubated at 37˚C, and the reaction was terminated by addition of SDS and EDTA to final concentrations of 0.5% (w/vol) and 25 mM respectively. The solution was subsequently treated with proteinase K for 1 hour at 37˚C and was then purified by phenol-chloroform extraction. To monitor cccDNA formation, the repair products were resolved on a DNA agarose gel containing 0.05 μg/ml ethidium bromide. The intensity of DNA bands was captured by Typhoon™ FLA 9500 (GE Healthcare Lifesciences) and subsequently quantified by ImageJ.

Reconstitution of cccDNA formation by purified murine and human proteins. cccDNA formation experiments with purified human factors were performed similarly to those experiments with nuclear extracts described above. The only exception was that 1.5 μM PCNA, 35 nM RFC, 20 nM POLδ, 100 nM LIG1, and 20 nM FEN-1 were used.

Simultaneous examination of the repair of individual lesions. This assay has been previously described in detail[40]. Briefly, the repair products at various time points were purified, and subsequently subjected to restriction enzyme digestion with AatII and PsiI (NEB). The digests were then resolved on a denaturing 5.5% (w/vol) urea-PAGE gel. DNA was subsequently transferred onto a positively charged nylon membrane (Sigma Aldrich), cross-linked to the membrane by UV irradiation (Stratalinker 1800), and each fragment was detected via digoxigenin (DIG) labeled probes.

Inhibition experiments with aphidicolin. Aphidicolin (Catalog number 102513, VWR) was dissolved in 100% DMSO (Sigma Aldrich) to a final concentration of 10 mM, which was subsequently diluted to 1 mM in buffer containing 20 mM HEPES-KOH (pH 8.0), 50 mM KCl, 5% glycerol, 1 mM DTT. Aphidicolin was added to cccDNA formation reactions to achieve a final concentration of 100 μM, 1% DMSO. Control treatments without aphidicolin contained 1% DMSO.

Immunoblotting analysis and antibodies. Protein samples were resolved by SDS-PAGE and transferred to a 0.2-μm nitrocellulose membrane (Biorad). Primary antibodies used were anti-PCNA (1:500 dilution, clone PC10, Santa Cruz Biotechnology), anti-POLD1 (1:1000 dilution, rabbit polyclonal, Proteintech), anti-FEN-1 (1:1500 dilution, mouse monoclonal, B-4, sc28355, Santa Cruz Biotechnology), anti-LIG1 (1:1000 dilution, 18051-1-AP, rabbit polyclonal, Proteintech), and anti-RFC4 (1:1000 dilution, clone 1320, GeneTex). Secondary antibodies used were DyLight 800-conjugated goat anti-Mouse IgG (1:3000 dilution, Thermo Fisher Scientific) and DyLight 680-conjugated goat anti-Rabbit IgG (1:3000 dilution, Thermo Fisher Scientific). For quantification, membranes were scanned with an Odyssey CLx Imager (Li-COR Biosciences). Quantification of blots was performed with ImageJ.

Supplementary figures

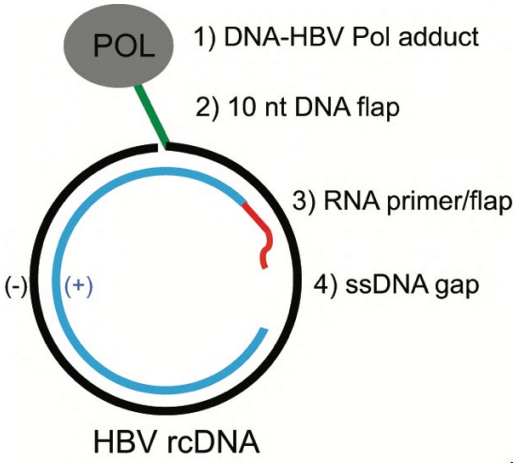

Fig. S1. Characteristics of HBV rcDNA structure.

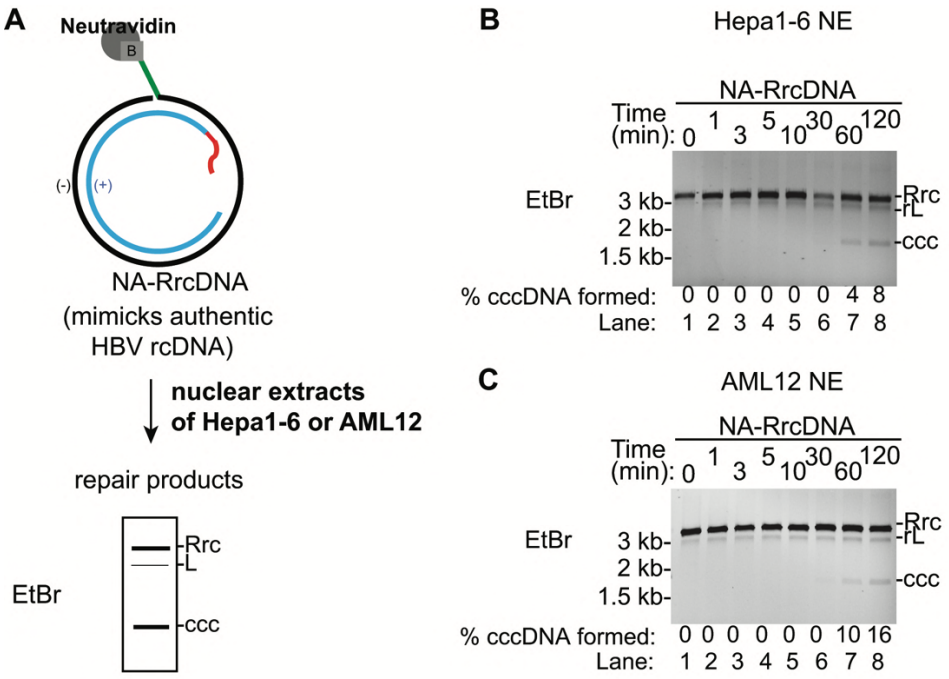

Fig. S2. Murine cell nuclear extracts support repair of NA-RrcDNA to form cccDNA. A) Schematics of cccDNA formation assay using murine nuclear extracts. **B)** Time course experiments showing the repair of NA-RrcDNA using murine nuclear extracts from Hepa1.6 and AML12. Rrc, RrcDNA; rL, recombinant linear RrcDNA; ccc, cccDNA.

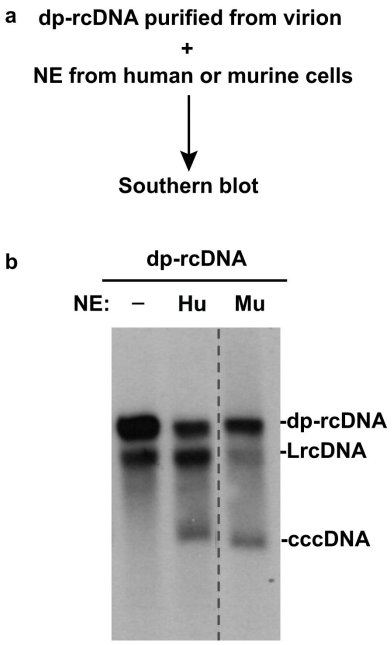

Fig. S3. Nuclear extract of Hepa1.6 supports cccDNA formation of virion derived rcDNA. A) Schematics of cccDNA formation assay using human and murine nuclear extracts and deproteinated rcDNA (dp-rcDNA) purified from HBV virion. **B)** cccDNA formation of virion derived dp-rcDNA by human HepG2 cell nuclear extract (protein concentration 10 mg/ml) and murine cell Hepa1.6 nuclear extract (protein concentration 20 mg/ml) was examined by Southern blot. LrcDNA, linear dp-rcDNA. Hu, human HepG2 extract; Mu, Murine Hepa1-6 extract; dashed line, superfluous lanes were removed.

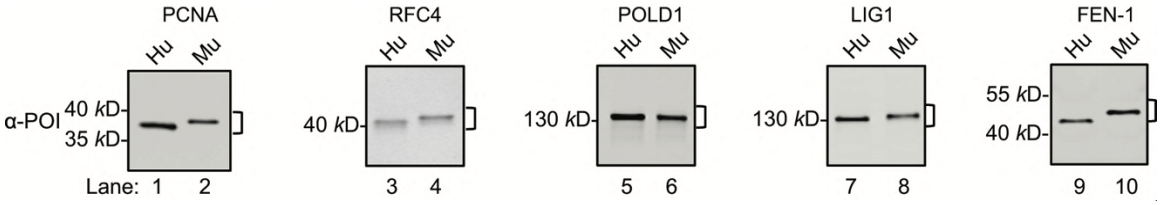

Fig. S4. Similar amounts of human (Hu) and murine (Mu) repair factors were used in cccDNA formation assays in Fig. 3 and Fig. S5. PCNA, RFC4, POLD1, LIG1 and FEN-1 levels were evaluated via western blot by antibodies reacting with both species.

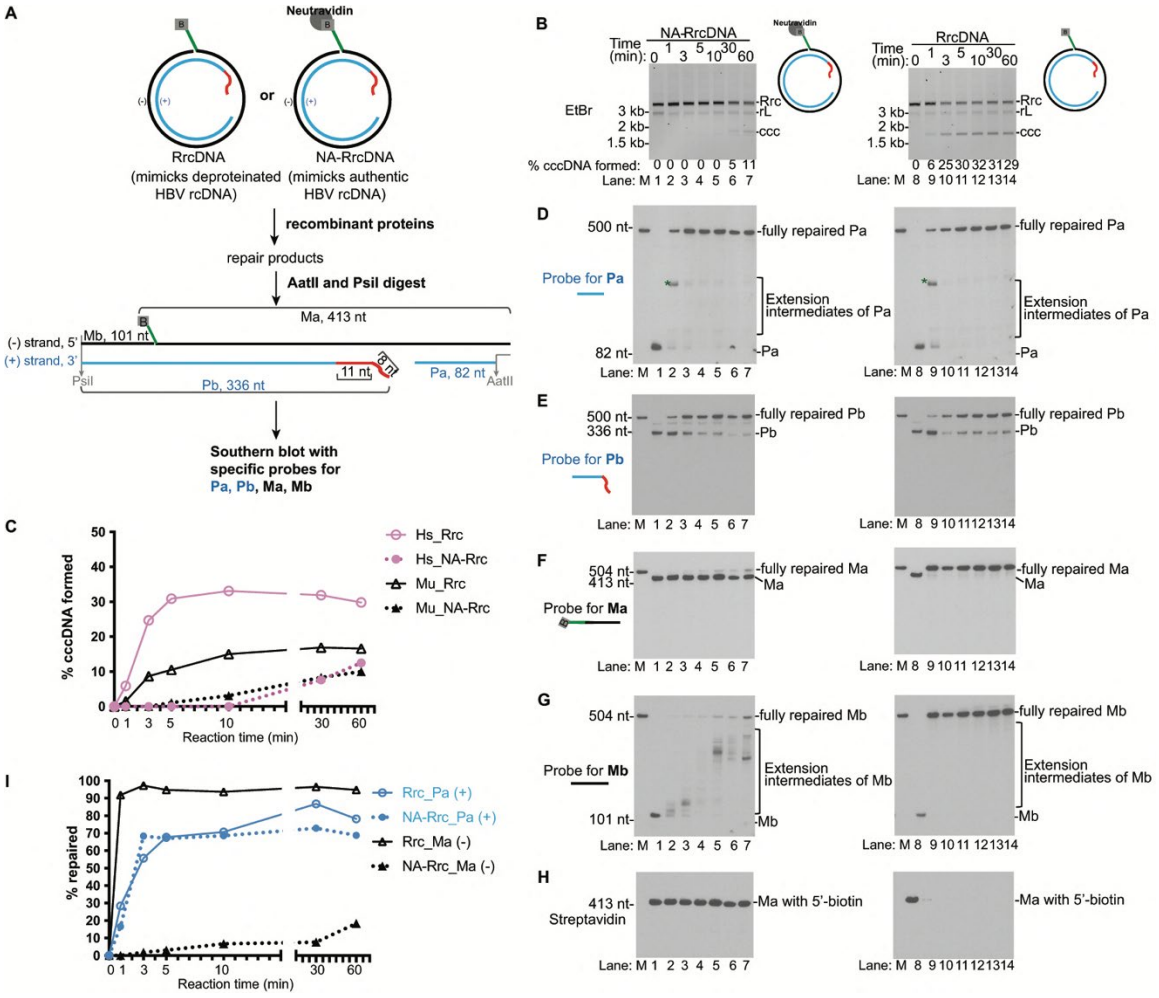

Fig. S5. Purified five human factors reconstitute repair of rcDNA to form cccDNA (related to Fig. 3). A) Schematics showing the biochemical assay that monitors the repair of all individual lesions of HBV rcDNA substrates RrcDNA and NeutrAvidin-RrcDNA (NA-RrcDNA) by five purified human factors. Green line, biotinylated flap; B, biotin; red line, RNA primer. **B)** Time course cccDNA formation assays with NA-RrcDNA (lanes 1–7) or RrcDNA (lanes 8–14) and five purified human factors, as depicted in (A). **(C)** "% cccDNA formed" from (B) was calculated as described in Fig. 3C and plotted. Hs, human; Mu, murine. **D)** The plus-strand Pa fragment repair is monitored by Southern blot. "*" indicates extension products of Pa that reach the 5′ end of Pb. **E)** The repair of plus-strand Pb fragment is monitored by Southern blot. **F-G)** Repair of minus-strand Ma and Mb fragments are monitored by Southern blot. **H)** Removal of biotin-harboring flap in Ma is detected by Streptavidin blot. **I)** The repair efficiencies of plus and minus strands are calculated from (E) and (F) and plotted. "% repaired" is calculated by dividing the band intensities of fully repaired Pa or Ma by the sum of the band intensities of unrepaired, intermediate, and fully repaired Pa or Ma. M, marker; Rrc, RrcDNA; rL, recombinant linear RrcDNA; ccc, cccDNA.

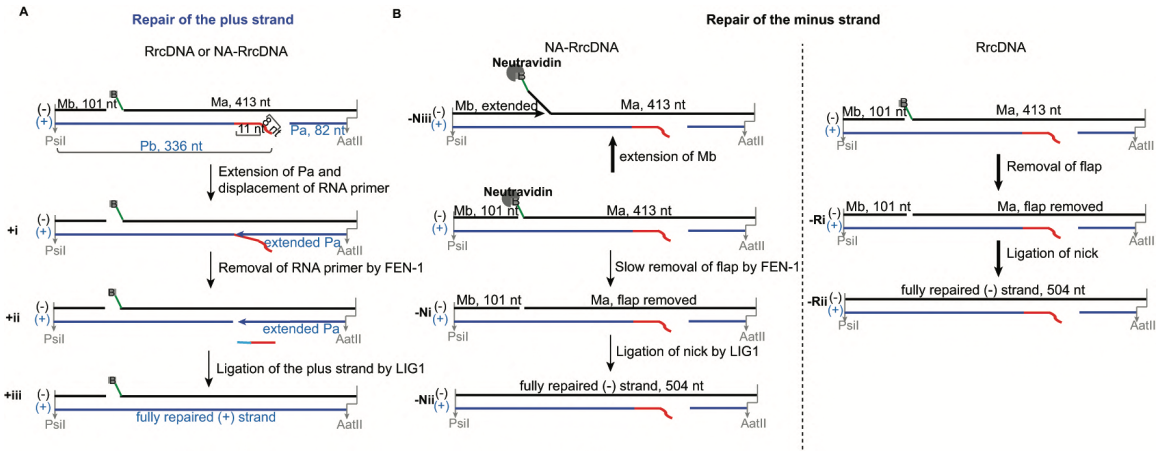

Fig. S6. Schematics of the HBV rcDNA repair process in the purified five-factor system. A) Model of the repair process of the plus-strand. **B)** Model of the repair process of the minus-strand. The repair efficiency of the minus-strand is influenced by the presence of a protein adduct. Left, when the protein adduct is present, DNA flap will be slowly removed by FEN-1, leaving only one nick on the minus-strand (-Ni), which is subsequently sealed by LIG1 (-Nii). The 3' end of the minus-strand ssDNA can be elongated by RFC-PCNA-POLδ and displaces the 5' end of the Mb fragment (-Niii). When RrcDNA is used as a template (right), the flap on the minus-strand will be rapidly removed by FEN-1, leaving a single nick (-Ri). This nick will then be ligated to complete the repair of the minus-strand (-Rii).

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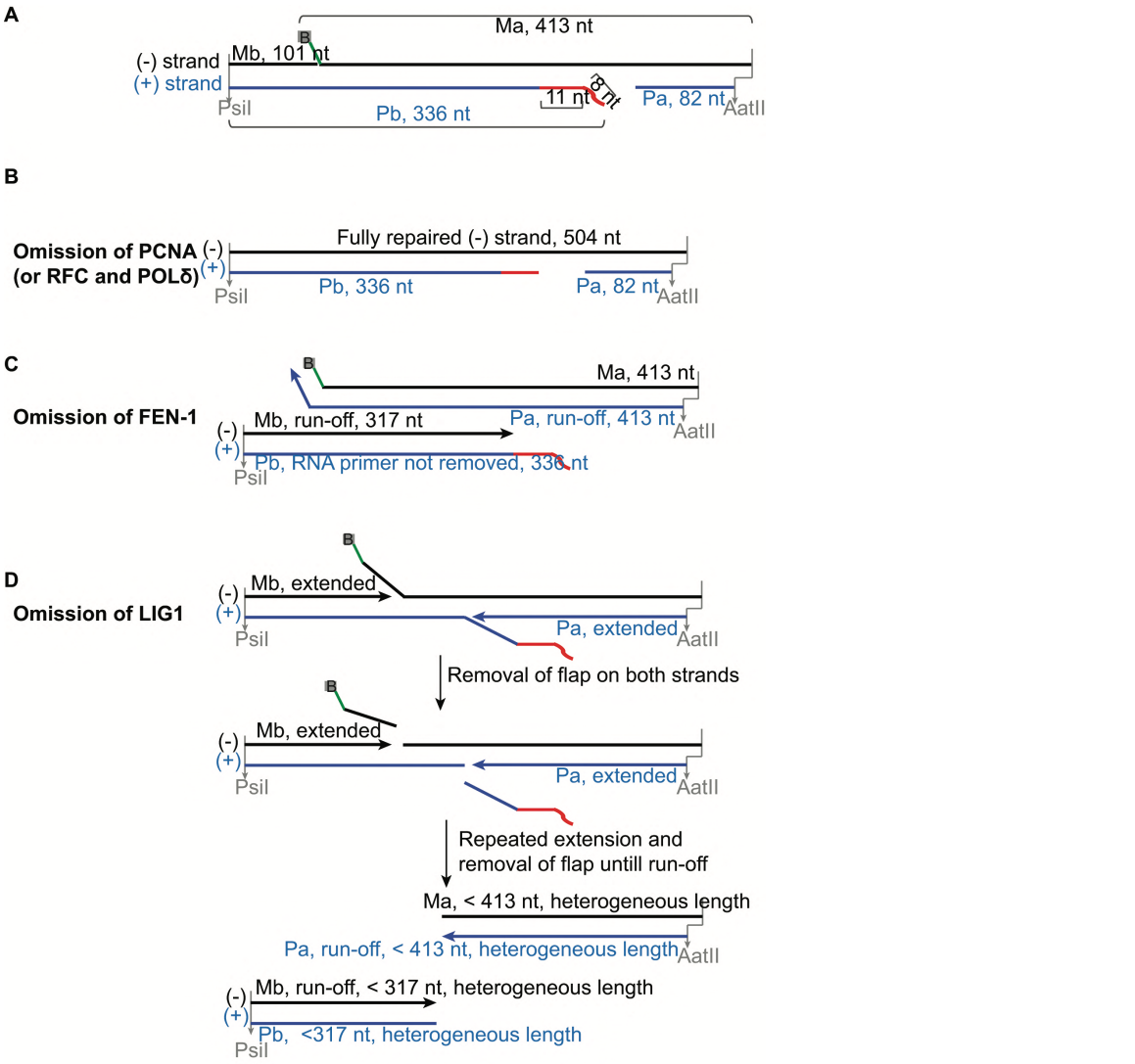

Fig. S7. Schematic of putative repair intermediates when individual repair factors are omitted. A) Schematic depicting the four fragments in unrepaired RrcDNA digested by AatII/PsiI. **B)** Theoretical repair intermediates when PCNA (or RFC and POLδ) is omitted. **C)** Repair intermediates of RrcDNA when FEN-1 is omitted. Without FEN-1, the DNA flap and RNA primer are not processed, and the 3' end of the plus- and minus-strands will be elongated by PCNA-POLδ until template run-off, linearizing the substrate. **D)** Repair intermediates when LIG1 is omitted. FEN-1 degrades Pb gradually as it gets displaced by Pa, therefore the final intermediate is similar to those when FEN-1 is omitted, but shorter in the Mb and Pb repair intermediates. Ma is also gradually degraded by FEN-1 as Mb displaces Ma. Therefore, the final intermediate is shorter than those in (C).

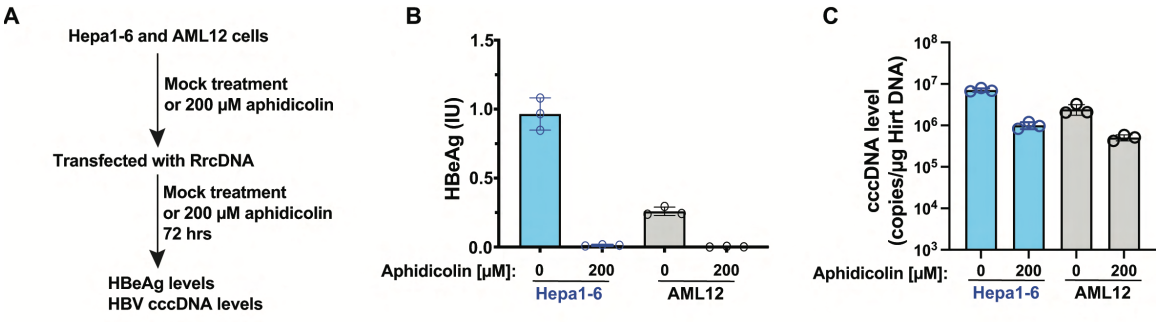

Figure S8. DNA polymerase inhibitor aphidicolin treatment reduces HBeAg, HBsAg, and cccDNA levels in Hepa1-6 and AML12 cells post transfection of recombinant rcDNA. A) Schematic of an experiment to examine the effects of aphidicolin on repair of HBV RrcDNA in Hepa1-6 and AML cells. **B)** HBeAg and HBsAg levels of Hepa1-6 and AML12 post transfection of RrcDNA are determined by ELISA. **C)** cccDNA levels of Hepa1-6 and AML12 post transfection of RrcDNA are determined by qPCR as in Fig. 1D.

Supplementary tables

Table S1. Protein sequence identity and similarity between murine and human proteins used in this study.

Proteins	Identity (compared to human orthologs)	Similarity (compared to human orthologs)
mPCNA	96.95%	98.47%
mFEN-1	96.58%	97.89%
mLIG1	84.10%	89.43%
mPOLD1	89.53%	94.22%
mPOLD2	94.26%	97.23%
mPOLD3	84.40%	90.81%
mPOLD4	83.33%	88.89%
mRFC1	82.02%	89.14%
mRFC2	91.55%	95.21%
mRFC3	97.20%	98.04%
mRFC4	90.96%	95.34%
mRFC5	94.13%	97.36%

Table. S2 Antibodies and antigen retrieval conditions for the fluorescent assay performed in this study

Sequence	Antigen Target	Species Origin	Manufacturer	Catalog	Primary Antibody Dilution	Antigen Retrieval (Ventana)	Fluorophore	Incubation Period	Incubation Temperature	Opal Dilution
1	HBV Core Ag	Rabbit	LSbio	C312204	1/20	CC1 (Tris)	Opal 480	32 minutes	37˚C	1/30
2	Ck17/19	Rabbit	Cell signaling	12434S	1/1000	CC1 (Tris)	Opal 570	32 minutes	37˚C	1/70
3	CD68	Rabbit	Abcam	ab12512	1/100	CC1 (Tris)	Opal 690	64 minutes	37˚C	1/150

Table. S3 Plasmids used in this study

Plasmid	Genotype	Source
pLW609	*pET15-6xhis-Flag-mPCNA*	this study
pLW610	*pET15-6xhis-Flag-mLIG1*	this study
pLW611	*pET15-mFEN-1-Flag-6xhis*	this study
pLW612	*pFastBac-Flag-mPOLD1, mPOLD4*	this study
pLW613	*pFastBac-mPOLD2, 6xhis-mPOLD3*	this study
pLW614	*pFastBac-mRFC2, mRFC3*	this study
pLW615	*pFastBac-mRFC4, 6xhis-mRFC5*	this study
pLW616	*pFastBac-Flag-mRFC1*	this study